

Outline of Production, a live virus vaccine shall meet the applicable requirements in this section.

(a) *Purity tests*—(1) *Bacteria and fungi*. Final container samples of completed product and comparable samples of each lot of Master Seed Virus shall be tested for bacteria and fungi in accordance with the test provided in § 113.27.

(2) *Mycoplasma*. Final container samples of completed product and comparable samples of each lot of Master Seed Virus shall be tested for mycoplasma in accordance with the test provided in § 113.28.

(3) *Avian Origin Vaccine*. Samples of each lot of Master Seed Virus and bulk pooled material or final container samples from each serial shall also be tested for:

(i) *Salmonella* contamination as prescribed in § 113.30; and

(ii) Lymphoid leukosis virus contamination as prescribed in § 113.31; and

(iii) Hemagglutinating viruses as prescribed in § 113.34.

(4) *Extraneous viruses*. Each lot of Master Seed Virus used to prepare live virus vaccine recommended for animals other than poultry shall meet the requirements for extraneous viruses as prescribed in § 113.55

(b) *Safety tests*. Samples of each lot of Master Seed Virus and final container samples of completed product from each serial or first subserial of live virus vaccine recommended for animals other than poultry shall be tested for safety in at least one species for which the vaccine is intended using methods prescribed in §§ 113.39, 113.40, 113.41, 113.44, and 113.45 or in a filed Outline of Production. The mouse safety test prescribed in § 113.33(a) shall also be conducted unless the virus or agent in the vaccine is inherently lethal for mice.

(c) *Virus identity test*. At least one of the virus identity tests provided in this paragraph or a suitable identity test prescribed in the filed Outline of Production shall be conducted on the Master Seed Virus and final container samples from each serial or first subserial of biological product.

(1) *Fluorescent antibody test*. The fluorescent antibody test shall be conducted using virus inoculated cells and uninoculated control cells. Cells shall be stained with fluorochrome con-

jugated specific antiserum. Fluorescence typical of the virus concerned shall be demonstrated in the inoculated cells. The control cells shall remain free of such fluorescence.

(2) *Serum neutralization test*. The serum neutralization test shall be conducted using the constant serum-decreasing virus method with specific antiserum. For positive identification, at least 100 ID₅₀ of vaccine virus shall be neutralized by the antiserum.

(d) *Cell Culture Requirements*. If cell cultures are used in the preparation of Master Seed Virus or of the vaccine, primary cells shall meet the requirements prescribed in § 113.51, cell lines shall meet the requirements prescribed in § 113.52, and ingredients of animal origin shall meet the applicable requirements in § 113.53.

(e) *Moisture content*. (1) The maximum moisture content in desiccated vaccines must be stated in the filed Outline of Production.

(2) Final container samples of completed product from each serial or subserial must be tested for moisture content in accordance with the test prescribed in § 113.29.

[39 FR 27430, July 29, 1974, as amended at 43 FR 49528, Oct. 24, 1978; 50 FR 1042, Jan. 9, 1985; 54 FR 19352, May 5, 1989. Redesignated at 55 FR 35562, Aug. 31, 1990; 60 FR 24549, May 9, 1995; 68 FR 57608, Oct. 6, 2003]

§ 113.301 Ovine Ecthyma Vaccine.

Ovine Ecthyma Vaccine shall be prepared from tissue culture fluids or virus-bearing tissues obtained from sheep that have developed ovine ecthyma following inoculation with virulent ovine ecthyma virus. Ovine Ecthyma Vaccine is exempt from the requirements prescribed in §§ 113.27 and 113.300(a), (b), and (c). Each serial shall meet the moisture requirements in § 113.300(e) and the special requirements prescribed in this section. Any serial found unsatisfactory by a prescribed test shall not be released.

(a) *Safety tests*. (1) Bulk or final container samples of completed product from each serial shall be tested for safety as prescribed in § 113.38.

(2) The prechallenge period of the potency test shall constitute a safety test. If unfavorable reactions attributable to the vaccine occur in either of

the vaccinates during the observation period, the serial is unsatisfactory.

(b) *Potency test.* Final container samples of completed product from each serial and each subserial shall be tested for potency using susceptible lambs. The vaccine shall be prepared as recommended for use on the label.

(1) Each of two lambs (vaccinates) shall be vaccinated by application of the vaccine to a scarified area on the medial surface of the thigh and observed each day for 14 days.

(2) The immunity of the two vaccinates and one or more unvaccinated lambs (controls) shall be challenged in the same manner as for vaccination, using the opposite thigh.

(3) If typical signs of ovine ecthyma, such as hyperemia, vesicles, and pustules do not develop on the controls during the first 2 weeks following challenge and persist for approximately 30 days, the test is inconclusive and may be repeated.

(4) If the vaccinates do not show a typical immune reaction, the serial is unsatisfactory: *Provided*, That, an initial active reaction with hyperemia which resolves progressively and disappears within 2 weeks, may be characterized as a typical immune reaction.

[39 FR 27430, July 29, 1974. Redesignated at 55 FR 35562, Aug. 31, 1990, as amended at 56 FR 66786, Dec. 26, 1991]

§ 113.302 Distemper Vaccine—Mink.

Distemper Vaccine—Mink shall be prepared from virus-bearing cell culture fluids. Only Master Seed Virus which has been established as pure, safe, and immunogenic shall be used for preparing the production seed virus for vaccine production. All serials of vaccine shall be prepared from the first through the fifth passage from the Master Seed Virus.

(a) The Master Seed Virus shall meet the applicable requirements prescribed in § 113.300 and the requirements prescribed in this section.

(b) The lot of Master Seed Virus shall be tested for extraneous viruses as follows:

(1) To detect virulent canine distemper virus, each of two distemper susceptible mink or ferrets shall be inoculated with 1 ml of the Master Seed Virus and observed each day for 21

days. If undesirable reactions occur in either test animal, the lot of Master Seed Virus is unsatisfactory.

(2) Master Seed Virus propagated in chicken embryos shall be tested for pathogens by the chicken embryo test prescribed in § 113.37 except lesions typical of distemper virus may be disregarded. If found unsatisfactory, the Master Seed Virus shall not be used.

(c) Each lot of Master Seed Virus used for vaccine production shall be tested for immunogenicity. The selected virus dose from the lot of Master Seed Virus shall be established as follows:

(1) At least 25 distemper susceptible mink shall be used as test animals. Blood samples shall be drawn from these animals and individual serum samples tested. The mink shall be considered susceptible if the results are negative at a 1:2 final serum dilution in a varying serum-constant virus neutralization test with less than 500 ID₅₀ of canine distemper virus. Other means of insuring susceptibility may be used if prior approval from Animal and Plant Health Inspection Service is received.

(2) A geometric mean titer of the dried vaccine produced from the highest passage of the Master Seed Virus shall be established before the immunogenicity test is conducted. At least 20 mink shall be vaccinated with a predetermined quantity of vaccine virus and at least 5 additional mink shall be held as unvaccinated controls. To confirm the dosage calculations, five replicate virus titrations shall be conducted on a sample of the vaccine virus dilution used.

(3) At least twenty-one days post-injection, the immunity of each of the vaccinates and the controls shall be challenged with the same size dose of virulent distemper virus and observed each day for 21 days.

(i) If at least 80 percent of the controls do not die or show severe signs of distemper, the test is inconclusive and may be repeated.

(ii) If at least 19 of 20, 27 of 30, or 36 of 40 of the vaccinates do not survive without showing clinical signs of distemper during the observation period, the Master Seed Virus is unsatisfactory.